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Cleavage of chromatin with methidiumpropyl-EDTA·iron(II)

(*Drosophila* DNA/intercalation/micrococcal nuclease/DNA structure/nucleosome positioning)

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ABSTRACT **Methidiumpropyl-EDTA·iron(II) [MPE·Fe(II)] cleaves double-helical DNA with considerably lower sequence specificity than micrococcal nuclease. Moreover, digestions with MPE·Fe(II) can be performed in the presence of certain metal chelators, which will minimize the action of many endogenous nucleases. Because of these properties MPE·Fe(II) would appear to be a superior tool for probing chromatin structure. We have compared the patterns generated from the 1.688 g/cm^3 complex satellite, 5S ribosomal RNA, and histone gene sequences of *Drosophila melanogaster* chromatin and protein-free DNA by MPE·Fe(II) and micrococcal nuclease cleavage. MPE·Fe(II) at low concentrations recognizes the nucleosome array, efficiently introducing a regular series of single-stranded (and some double-stranded) cleavages in chromatin DNA. Subsequent S1 nuclease digestion of the purified DNA produces a typical extended oligonucleosome pattern, with a repeating unit of ca. 190 base pairs. Under suitable conditions, relatively little other nicking is observed. Unlike micrococcal nuclease, which has a noticeable sequence preference in introducing cleavages, MPE·Fe(II) cleaves protein-free tandemly repetitive satellite and 5S DNA sequences in a near-random fashion. The spacing of cleavage sites in chromatin, however, bears a direct relationship to the length of the respective sequence repeats. In the case of the histone gene sequences a faint, but detectable, MPE·Fe(II) cleavage pattern is observed on DNA, in some regions similar to and in some regions different from the strong chromatin-specified pattern. The results indicate that MPE·Fe(II) will be very useful in the analysis of chromatin structure.**

With our current appreciation of the nucleosome as the fundamental unit of chromatin condensation (1–3), it has been pertinent to ask whether or not there is a functional requirement for a particular nucleosomal array. This aspect of chromatin structure has been most often expressed in the concept of specific nucleosome positioning (or "phasing") at a few or many loci of the eukaryotic genome, perhaps in a cell-, tissue-, or development-specific manner. Possible advantages of such positioning have been envisaged by many investigators, although no positive evidence for its actual functional importance *in vivo* has yet been presented. Numerous studies arguing for a specific or, conversely, for a random distribution of nucleosomes have been reported, and these have been reviewed (4–7). Many of these experiments have utilized micrococcal nuclease for generation of nucleosomal arrays. The DNA is purified subsequent to the nuclear digestion and the cleavage sites are mapped by reference to well-characterized restriction sites. Unfortunately, micrococcal nuclease has a marked sequence preference and introduces cleavages into purified DNA at quite specific and reproducible positions (8, 9). In some cases these occur at exactly the same sites in chromatin, leading to uncertainty concerning which is chromatin specific and which is purely sequence specific. As an example, the majority of the cuts introduced across a 12-kb region of heat shock locus 67B1 in *Drosophila* chromatin are identical with those seen in purified DNA (10). Such a result makes it difficult to derive meaningful conclusions about the specific placement of nucleosomes (or lack thereof). In contrast, the use of micrococcal nuclease in a careful comparison with protein-free DNA control digests has permitted unambiguous mapping of nucleosomes around a cluster of inactive *Xenopus* tRNA genes (11) and around the centromere of chromosomes III and XI of yeast (12). Notwithstanding this, a means of generating nucleosome arrays with a sequence-neutral DNA cleavage reagent would be highly desirable.

A nonenzymatic method for chromatin structure analysis utilizing the DNA-cleaving 1,10-phenanthroline-cuprous complex was recently reported (13). This reagent recognized the nucleosomal structure of chromatin and produced a cleavage pattern based on a regularly repeated unit. However, cleavages virtually identical to those produced by micrococcal nuclease were introduced into protein-free DNA, an observation interpreted in terms of a common recognition of secondary structural characteristics of the DNA double helix. The synthesis of another DNA-binding/DNA-cleaving molecule has recently been described (14). Methidiumpropyl-EDTA (MPE) cleaves DNA efficiently in the presence of Fe(II), O_2, and reducing agents such as dithiothreitol (14). Importantly, MPE·Fe(II) cleaves DNA with low sequence specificity and is a useful tool for determining the multiple binding sites of drugs on native DNA (15). We report here that MPE·Fe(II) will cleave nuclear chromatin into oligonucleosomes under reaction conditions in which cleavage by endogenous nucleases can be minimized. The nucleosomal products generated are similar in size to those from micrococcal nuclease digestion and appear to be due to highly preferential cleavage in linker DNA. A comparison with the patterns generated on protein-free DNA for a number of sequences indicates that MPE·Fe(II), although not entirely sequence neutral, will prove useful in nucleosomal mapping studies.

MATERIALS AND METHODS

Nuclear Digestions. Nuclei were isolated from 6- to 18-hr *Drosophila melanogaster* embryos as described (16) except that the final centrifugation was through 1.7 M sucrose. Nuclei were resuspended in the relevant buffer and digestions were performed at 25°C. Micrococcal nuclease (Worthington) digestions of nuclei at 1×10^9 per ml were for 3 min at various enzyme concentrations as described (10, 13). The general protocol for digestions with MPE·Fe(II) is as follows, with specific details noted in the figure legends. Nuclei were resuspended in diges-

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Abbreviations: MPE, methidiumpropyl-EDTA; kb, kilobase(s); bp, base pair(s).

tion buffer (15 mM Tris·HCl, pH 7.4/15 mM NaCl/60 mM KCl/0.25 M sucrose) at 2×10^9 per ml. The MPE·Fe(II) complex was prepared separately by mixing equimolar quantities of MPE (synthesized as described in ref. 14 and stored as a 1 mM stock solution in water at −20°C) and freshly prepared ferrous ammonium sulfate and diluting to the required concentration with digestion buffer. Just prior to initiating the reaction, freshly dissolved dithiothreitol was added to the MPE·Fe(II) complex to a final concentration of 2 mM. In some cases, hydrogen peroxide, EDTA, or both were included in the mixtures for MPE·Fe(II)-mediated nuclear digestion as noted in the text. After equal volumes of nuclear suspension and MPE·Fe(II) had been mixed, incubations were performed at 25°C for various periods. Aliquots of reaction mixture were withdrawn and mixed with 0.1 vol of 50 mM bathophenanthrolinedisulfonate (4,7-diphenyl-1,10-phenanthrolinedisulfonate, Sigma) to stop the cleavage reaction. $NaDodSO_4$ and EDTA were added to 0.5% and 12.5 mM, respectively. DNA fragments from both micrococcal nuclease and MPE·Fe(II) digestions were purified and isolated by standard procedures (16).

Analysis of Digestion Products. Agarose (Seakem) gel electrophoresis, Southern transfers, and hybridization of nick-translated DNA fragments were conducted as described (10, 13, 16). Recombinant cosmid mDm107 [which contains approximately 9 kilobases (kb) of the *Drosophila* 1.688 g/cm^3 satellite; see ref. 17], and plasmids 12D1 (18) and B5 [a *Bam*HI/*Bam*HI subclone, prepared by D. W. Miller, of the plasmid cDm500 (19)] were grown under P1–EK1 conditions in accordance with National Institutes of Health guidelines. Agarose gels were stained in ethidium bromide at 0.5 μg/ml as required. Restriction enzymes were purchased from New England BioLabs or Bethesda Research Laboratories, and digestions were performed according to the supplier's recommendations. S1 nuclease (Boehringer/Mannheim) digestions were performed in 3 mM zinc sulfate/0.6 M sodium chloride/30 mM sodium acetate, pH 4.6, at the indicated temperature. S1 nuclease units are those quoted by Boehringer (i.e., 30-min units as opposed to the more usual 1-min units). Reactions with S1 nuclease were terminated as for micrococcal nuclease digestions.

RESULTS

The MPE·Fe(II) complex efficiently introduces double-stranded cleavages at regularly repeating intervals in chromatin from *Drosophila* embryo nuclei as shown in Fig. 1*A*. In a comparison with the oligonucleosomal patterns produced by micrococcal nuclease the specificity of the MPE·Fe(II) cleavage reaction on chromatin appears at least as great, as deduced from the ethidium bromide staining pattern. Characterization of the reaction led to some useful observations. First, the reaction was not inhibited significantly by addition of EDTA at concentrations of 10 mM or less. The presence of submillimolar EGTA can also be tolerated, although there may be some slight inhibition relative to a control digest. While it was not possible to prepare a highly reactive MPE·Fe(II) complex in solutions already containing EGTA, the complex, once formed, apparently remains active if subsequently added to EGTA-containing nuclear suspensions. Second, the reaction is very effectively stopped by addition of bathophenanthroline, a highly specific iron-complexing agent. Third, the digestion shown in Fig. 1*A* was performed at a ratio of MPE·Fe(II) to DNA base pairs of approximately 1:15. Addition of millimolar quantities of hydrogen peroxide enhances the rate of reaction quite effectively, and it is possible to work at concentrations of fresh MPE·Fe(II) as low as 1 μM. Under these conditions there is probably considerably less than 1 molecule of the intercalator in the nucleus for every 200 bp of DNA in chromatin.

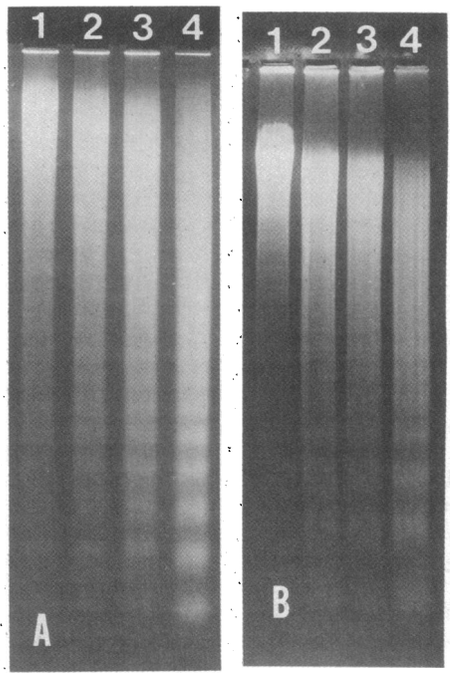

FIG. 1. Electrophoretic analysis of cleavage fragments from MPE·Fe(II) nuclear digestion. (*A*) Lanes 1–3 are samples digested with MPE·Fe(II) at 50 μM for 20, 25, and 30 min, respectively. Lane 4 is a 3-min digestion with micrococcal nuclease at 23.5 units/ml. (*B*) Lane 1, 5-min digestion with MPE·Fe(II) as in *A* but with 0.5 mM H_2O_2 added. Lanes 2–4, 2-μg portions of this sample were digested in 20 μl of S1 buffer at 0°C for 30 min with 100, 250, and 1,000 units of S1 nuclease, respectively. Samples were electrophoresed on 1.2% agarose and stained with ethidium bromide.

Close inspection of Fig. 1*A* reveals that the nucleosome bands migrate slightly slower than the corresponding oligomers produced by micrococcal nuclease. This might be caused by extensive single-stranded tails, internal single-stranded regions within the double-stranded fragments, or both. Such a result would not be unexpected owing to the predominantly single-stranded nicking activity observed for the MPE·Fe(II) complex (14). In Fig. 1*B*, the purified DNA from a suitable early digestion point of a MPE·Fe(II) digestion series of nuclear chromatin was incubated with S1 nuclease, and the resulting fragments are displayed on an agarose gel. The effect is to change the distribution of fragment sizes quite dramatically, a result consistent with the notion that significant numbers of nicks or single-stranded gaps are present in the product from nuclear digestion with MPE·Fe(II). Under the salt conditions used, S1 should not introduce a significant number of new nicks, and this has been confirmed on denaturing gels (data not shown). An important conclusion from Fig. 1*B* is that the nicks or gaps occur in a regularly repeating fashion (presumably in linker DNA), because otherwise the nucleosomal pattern would smear and become unrecognizable after treatment of the DNA with S1 nuclease. Such smearing is not observed, suggesting that MPE·Fe(II) recognizes the linker region between nucleosome cores with high specificity.

Fig. 2 shows MPE·Fe(II) digests of both nuclear chromatin and protein-free DNA compared with similar micrococcal nuclease digests analyzed for two repetitive sequences of the *Drosophila* genome. In Fig. 2*A*, the 1.688 g/cm^3 satellite-specific sequences are displayed. Both reagents show a prominent nucleosomal ladder derived from the chromatin samples. The ef-

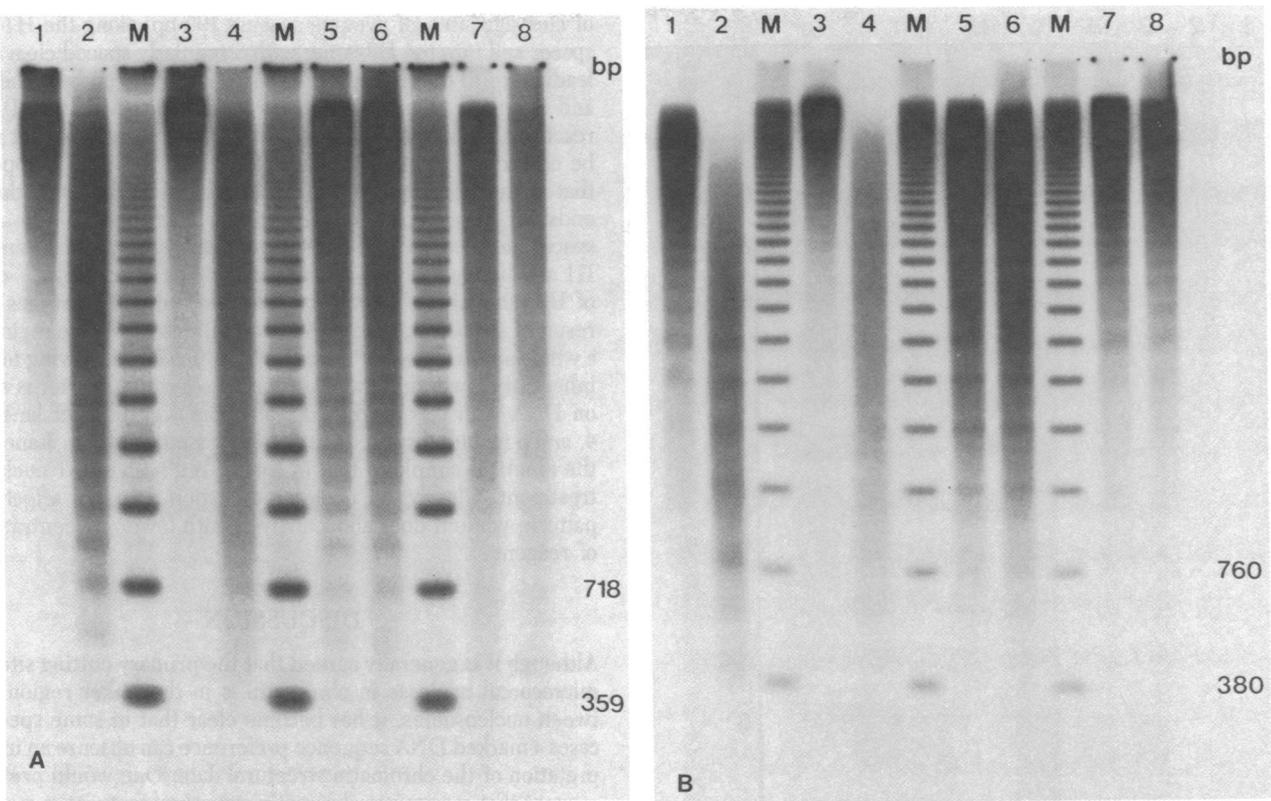

FIG. 2. Comparison of MPE·Fe(II) and micrococcal nuclease activity on chromatin and DNA for 1.688 g/cm^3 satellite and 5S ribosomal RNA sequences. Lanes 1–4 are MPE·Fe(II) digests with (lanes 2 and 4) and without (lanes 1 and 3) subsequent S1 nuclease treatment. Lanes 5–8 are micrococcal nuclease digests with (6 and 8) and without (5 and 7) S1 nuclease treatment. Lanes 1 and 2 are chromatin digests with MPE·Fe(II) at 50 μM for 5 min. Lanes 5 and 6 are chromatin digests with micrococcal nuclease at 11.7 units/ml for 3 min. DNA digestions were at 140 μg/ml with 10 μM MPE·Fe(II), 0.5 mM H_2O_2, and 1 mM dithiothreitol for 4 min at 25°C (lanes 3 and 4) or at 500 μg/ml with micrococcal nuclease at 12 units/ml for 1 min at 25°C (lanes 7 and 8). S1 nuclease digestions were as in Fig. 1, but using 1,250 units of S1 nuclease in S1 buffer for 30 min at 0°C. Samples were subjected to electrophoresis on a 1.2% agarose gel, transferred to nitrocellulose, and hybridized to satellite (*A*) and (by reusing) to 5S ribosomal sequences (*B*). M, partial *Hae* III digest of purified genomic DNA as size markers (359 bp in *A*; 380 bp in *B*).

fect of S1 nuclease on the MPE·Fe(II) digests is as expected from Fig. 1*B*; corresponding digestion with S1 nuclease of the micrococcal nuclease-treated samples shows no such diminution in fragment sizes, as expected from the double-stranded nature of these enzymic cleavages. S1 digestion also increases the mobility of the MPE·Fe(II)-derived oligonucleosome fragments to match that of those produced by micrococcal nuclease. The correspondence between even-numbered oligonucleosome bands and the sequence repeat [359 base pairs (bp)] is virtually exact, extending up to 20-mers or more. In contrast, MPE·Fe(II) digestion of protein-free satellite DNA sequences produces a smear, whereas micrococcal nuclease yields faint sequence preferential cleavages (visible in the original autoradiogram) in the satellite DNA twice per sequence repeat. The 5S ribosomal RNA sequences are displayed in Fig. 2*B* (the filter of Fig. 2*A* was washed in 0.1 M NaOH and the DNA was rehybridized). The sequence-preferred cleavage sites in naked DNA are very evident for micrococcal nuclease. In this instance, there is some very slight (detectable in the original autoradiogram) preferential cleavage in the 5S sequences by MPE·Fe(II). Distinctive differences are apparent in the chromatin digests. MPE·Fe(II) produces a nucleosomal ladder with the typical nucleosomal breadth apparent in the bands. The pattern is based upon a dinucleosomal repeat; odd-numbered oligomer bands cannot be seen with definition beyond the pentamer. In general, the length of the even-numbered oligomer bands is very close to an integral multiple of the 5S sequence repeat (380 bp). The enzymic pattern appears superficially similar but is rather different in its particular details. The even-numbered bands of the ladder are very sharp and appear to be more characteristic of those expected from a DNA sequence-preferential cleavage. In addition, the few small odd-numbered bands that can be discerned are of unusual size and do not fall in the position expected for a regular nucleosomal array. Thus for both the satellite and 5S gene sequences the products of MPE·Fe(II) digestion form a nucleosomal array showing spacing corresponding to the DNA sequence repeat.

The histone genes of *Drosophila* represent an interesting locus for nucleosome positioning studies. The major 5-kb repeating unit (19) allows effective use of the indirect end-labeling technique (20, 21). A previous study by others using micrococcal nuclease showed that a unique and specific positioning of nucleosomes occurred across the 1.2-kb H1–H3 spacer. Across the genes themselves the chromatin cleavage pattern was virtually identical with that derived from protein-free DNA (22), with prominent cutting sites in spacers but relatively less cutting in the genes. Fig. 3 shows a comparison of MPE·Fe(II) and micrococcal nuclease cleavage sites for both chromatin and protein-free DNA. A *Bgl* II/*Bam*HI probe from within the H1 gene was used, and mapping from the *Bgl* II site is shown. MPE·Fe(II) exhibited some faint, but clearly distinguishable, sequence-preferential cleavage in the DNA controls. However, in many regions, the MPE·Fe(II) cleavages on chromatin appear to be defined by chromatin structure. Where there is a correspondence in the patterns, the relative intensity of cleavage is much higher in the chromatin samples. The pattern in

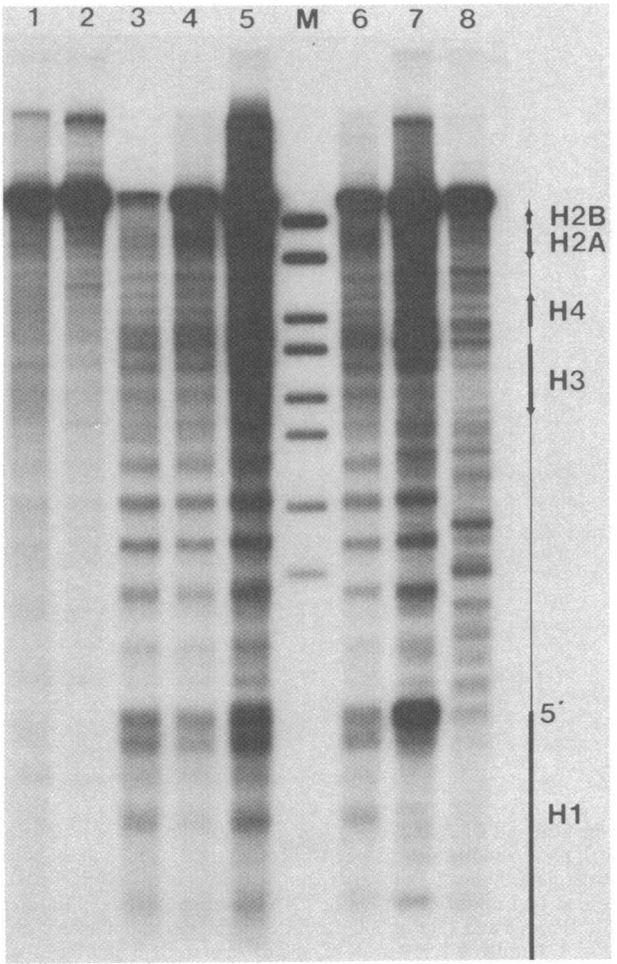

FIG. 3. Mapping chromatin structure in the histone gene repeat. A comparison of MPE·Fe(II) (lanes 1–6) and micrococcal nuclease (lanes 7 and 8) digestion of chromatin (lanes 3–7) and purified DNA (lanes 1, 2, and 8). Chromatin samples in lanes 3, 4, and 5 were treated with S1 nuclease as in Fig. 2. MPE digestions of nuclei were at 5 μM MPE·Fe(II) for 5 min with 0.5 mM H_2O_2 (lanes 3 and 6), for 10 min with no H_2O_2 (lane 4), and at 20 μM MPE·Fe(II) for 7.5 min with 0.5 mM H_2O_2 and 1 mM EDTA/0.1 mM EGTA (lane 5). MPE·Fe(II) digestions of DNA were as in Fig. 2 for 4 min (lane 1) or 1 min (lane 2). Nuclei were digested with micrococcal nuclease at 8.8 units/ml for 3 min; DNA digestion was as for Fig. 2 (lane 7). All DNA samples were completely digested with *Bgl* II, and 4-μg samples were subjected to electrophoresis on a 1.6% agarose gel, blotted to nitrocellulose, and hybridized to the small *Bgl* II/*Bam*HI fragment of B5, which was excised from a gel as described (13). M, pBR322 size markers.

chromatin produced by micrococcal nuclease has certain unique features, particularly in the H1–H3 spacer, but also often mimics the intense patterns seen in DNA (compare lanes 7 and 8). The comparison between the DNA controls for the two reagents (lanes 1, 2, and 8) reveals some interesting features. The patterns themselves are often very different in detail, and the pattern generated by using MPE·Fe(II) is inherently much weaker in the intensity of the cleavages compared to the prominent cuts often produced by the enzyme. In contrast, the patterns generated on digestion of chromatin by the two reagents show much more homology. Cleavage with MPE·Fe(II) reveals additional information, particularly over the genes. Two chromatin-specific cleavages (of approximately 155-bp spacing) are located on the H1 gene distal to the pair of strong hypersensitive sites at the 5′ end (the latter are spaced approximately 75 bp apart). In addition, there appears to be precise positioning of cleavage sites (of average spacing 190 bp) along the H1–H3 spacer and into the H3 gene, with 3 regularly spaced cleavages leading up to the hypersensitive sites at the 5′ ends of the H3 and H4 genes. Our measurements show that MPE·Fe(II) can react to singularities in the protein–DNA interaction that may be characteristic of particular activity states—e.g., it appears that the reagent recognizes the hypersensitive sites at the 5′ ends of genes. One observes smaller than nucleosome-sized spaces between the pair of hypersensitive sites at the 5′ end of H1 and between the hypersensitive sites flanking the 5′ ends of H2A and H2B. The interpretation of unique cleavages as a result of specific protein–DNA associations across the region as a whole appear possible from the MPE·Fe(II) data owing to the inherently lower and more uniform background patterns seen on DNA with this reagent. Finally, the digestions of lanes 3, 4, and 5 included subsequent S1 nuclease treatment. Lane 6 is the identical sample to that in lane 3, but without S1 nuclease treatment. Clearly, S1 nuclease digestion does not affect the pattern, while it does allow analysis with lower concentrations of reagent.

DISCUSSION

Although it is generally agreed that the primary cutting site for micrococcal nuclease in chromatin is in the linker region between nucleosomes, it has become clear that in some specific cases a marked DNA sequence preference can obscure an interpretation of the chromatin structural data. One would prefer a reagent that recognizes chromatin structural features (e.g., the linker region between nucleosomes) but is relatively free from responses to DNA sequence. As a probe of chromatin structure the synthetic MPE·Fe(II) complex appears to offer some distinct advantages over micrococcal nuclease. It seems likely that the chromatin specificity of MPE·Fe(II) stems from intercalative binding of the methidium portion of the complex to DNA. The results shown in Figs. 1 and 3 suggest that linker DNA is indeed the site of regularly repeated cleavage by MPE·Fe(II). The data of Fig. 3 demonstrate an identical pattern of cleavage sites at approximately 190-bp intervals for chromatin in the H1–H3 spacer for both MPE·Fe(II) and micrococcal nuclease. In addition, in this region the micrococcal nuclease data appear to be unambiguous with respect to protein-free DNA cleavage, indicating that the primary specificity of MPE·Fe(II) lies in preferential binding to internucleosomal linker DNA. Intercalators that can be bound covalently to chromatin (e.g., 4,5′,8-trimethylpsoralen) appear to locate highly preferentially in linker regions (23, 24). The evidence available therefore points strongly toward the primary site of action for MPE·Fe(II) cleavage of chromatin residing in the internucleosomal linker.

An important feature of the reaction for chromatin studies is that, once formed, the MPE·Fe(II) complex is active in the presence of EDTA and low concentrations of EGTA. Nuclei can be isolated and digested in the presence of these metal chelators, thus minimizing the action of any endogenous metal-dependent nucleases. It is also apparent that only low overall extents of digestion are needed, because excellent nucleosomal ladders can be produced by secondary S1 nuclease digestion of the purified DNA in high salt concentrations and at low temperature, conditions in which only single-stranded tails, DNA opposite nicks, or single-stranded gaps are cleaved. Thus low ratios of MPE·Fe(II) to DNA base pairs can be utilized, which should preclude any major distortion of the native chromatin structure at a given locus.

The regularly repeated nucleosomal packaging of the 1.688 g/cm^3 *Drosophila* satellite is clearly visible in Fig. 2*A* from the MPE·Fe(II) digestions. The protein-free DNA cleavage pat-

terns show a slight cleavage preference twice per sequence repeat for micrococcal nuclease, whereas this is not discernible for MPE·Fe(II). The very precise register between even-numbered nucleosomal bands and the sequence repeat (359 bp; ref. 25), up to the highest oligomers that can be observed, allows an inference that the chromatin structure is constrained (if not controlled) by the nature of the DNA sequence repeat. This satellite is, however, very heterogeneous in terms of single base changes and attendant restriction site heterogeneity (refs. 17 and 25 and unpublished observations) and numerous attempts to map nucleosome positioning have been thwarted by this. The data presented indicate that the nucleosomal repeat is precisely defined in a manner related to the DNA sequence but do not address the question of unique positioning with respect to the sequence.

Micrococcal nuclease has clearly defined cleavage preferences on the 5S DNA sequence repeat. The comparison of micrococcal nuclease and MPE·Fe(II) cleavage of chromatin is interesting. Both show for the most part a prominent cleavage site occurring every second nucleosome, implying a protection of the linker region in every other nucleosome. However, the micrococcal nuclease bands are quite sharp compared to normal nucleosomal bandwidths, raising the possibility that they may be sequence specified, at least in part. The MPE·Fe(II)-derived nucleosomal bands are of a more typical width. The exact position of the cleavages generated with MPE·Fe(II) remains to be investigated.

The experiment on the histone gene repeat of *Drosophila* demonstrates the ability of MPE·Fe(II) to detect unique protein–DNA associations and provides some further information on a region where micrococcal nuclease has been of value (22). MPE·Fe(II) responds to some features of the specific chromatin structure that are probably not related in a direct way to nucleosome placement—e.g., the hypersensitive sites at the 5′ ends of the genes, previously detected with DNase I (22). The extended array of regularly spaced cleavages across the H1–H3 spacer seems most likely to be the result of precisely positioned nucleosomes spaced at approximately 190-bp intervals. The two regularly spaced (155-bp) sites in the H1 gene immediately distal to the 5′ hypersensitive region may represent a compact nucleosome spacing or may be a manifestation of some other form of protein–DNA interaction. These observations, together with the more defined cleavages produced by MPE·Fe(II) in chromatin across the other regions encoding transcripts (compared to micrococcal nuclease) indicate that MPE·Fe(II) is a valuable addition to the spectrum of reagents used for chromatin structural studies. Such a statement is not unqualified because it is apparent from the DNA controls in Fig. 3 that MPE·Fe(II) displays some low-level DNA sequence specificity and that in certain regions similarities between the DNA and chromatin-specific cleavage patterns exist. Two important points can be made. First, the chromatin-specific patterns detected by the two reagents appear to be substantially similar for this locus. Second, the protein-free DNA patterns are quite different from one another, both in the positions of the preferred cleavage sites and in their relative intensities, MPE·Fe(II) having much less distinct sequence preferences than micrococcal nuclease. In particular, in regions other than the H1–H3 spacer, the cleavages for micrococcal nuclease are similar for chromatin and protein-free DNA, both in intensity and location. This is not true for MPE·Fe(II) cleavages.

Analyses of the cleavage behavior of MPE·Fe(II) at the sequencing level on fragments of pBR322 (15) or *Drosophila* 5S ribosomal sequences (unpublished observations) have shown a fairly uniform cleavage at each succeeding base, although modest variations in the intensity of the cleavages are seen. However, a pattern is observed for the DNA of the *Drosophila* histone locus; this may be related to differences in the secondary structure of the DNA recognized by the reagent (15). Mapping from the other end of the histone repeat with MPE·Fe(II) shows a similar degree of correspondence between the DNA and chromatin structural patterns, with the intensity of the latter cleavages always much increased (data not shown). Whether such patterns in DNA structure have relevance for the final chromatin structure adopted by these sequences is unknown.

It seems apparent that MPE·Fe(II) will be a very useful reagent for analysis of chromatin structure. At the present level of resolution it appears to provide more definitive data than micrococcal nuclease on nucleosome distributions across the three loci examined. Other features of the protein–DNA interaction are also revealed.

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